

Managing earthworm casts (Oligochaeta: Lumbricidae) in turfgrass using a natural byproduct of tea oil (*Camellia* sp.) manufacture

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Abstract

BACKGROUND: Earthworm casts are a worldwide problem on golf courses and sports fields when they disrupt the playability, aesthetics and maintenance of closely mowed playing surfaces. Currently, no pesticides are labeled for earthworms in the United States. Tea seed pellets (TSPs), a saponin-rich byproduct of *Camellia oleifera* Abel oil manufacture, were tested for expelling earthworms and reducing casts on creeping bentgrass turf. The fate of expelled worms, methods for removing them and impacts on pest and beneficial arthropods were also evaluated.

RESULTS: Application of TSPs at 2.93 kg 100 m⁻², followed by irrigation, quickly expelled earthworms from the soil. A single application reduced casts by 80–95% for at least 5 weeks. Mowing or sweeping removed expelled earthworms from putting green surfaces. Most expelled earthworms burrowed down when transferred to untreated turf, but few survived. Bioassay-guided fractionation confirmed the vermicide activity results from a mix of saponins. TSPs did not reduce the abundance of beneficial soil arthropods, nor did they control black cutworms or white grubs in treated turf.

CONCLUSION: TSPs are an effective botanical vermicide that could be useful for selectively managing earthworm casts on closely mowed turfgrass. They might also be used to suppress earthworms in grassy strips alongside runways to reduce bird strike hazard at airports.

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Keywords: vermicide; *Camellia oleifera*; saponins; Lumbricidae; *Agrostis stolonifera*

1 INTRODUCTION

Earthworms (Oligochaeta: Lumbricidae) play a vital role in natural and managed grasslands, where their burrowing and feeding activity stimulates microbial activity, increases plant nutrient availability, breaks down and mixes surface litter into the soil and enhances infiltration of water and gases.^{1,2} Earthworm activity also benefits lawns, golf courses, sports fields and other managed turf sites by alleviating soil compaction,³ facilitating decomposition and recycling of organic matter and alleviating build-up of excessive thatch.⁴

Earthworms can be a serious problem, however, when their casts disrupt the playability and aesthetics of closely mowed playing surfaces.^{5–8} Casting occurs when worms deposit their soil-rich fecal matter as small mounds, or casts, on the surface.² Casts result in uneven, muddy playing surfaces and adversely affect ball roll. When smeared or compacted by tires or foot traffic, they may smother the grass and cause problems with water infiltration.⁶ Casts also provide ideal seedbed conditions for weed establishment.⁹ They blunt mower blades set at low cutting heights, and may be so numerous that putting greens cannot be mowed without first dispersing the casts using a brush or steel drag. Casting activity is especially prolific during cool, moist periods in autumn and spring.^{6,7} Earthworms in grass aprons of runways and taxiways or moving onto pavement after rains may

attract robins, starlings and other birds, increasing bird strike hazard at airports.^{10–12}

No pesticides are labeled for earthworm control in the United States. Research on alternative methods for managing earthworms and casts has focused on soil acidification, clipping removal to limit food resources or topdressing with angular sands or abrasive aggregates.^{6,7,13,14} Although these methods may sometimes reduce casting,¹⁴ they generally are not consistent or effective enough to be relied upon by green-keepers, are impractical or can adversely affect the turf. Physical removal of casts by brushing, switching or dragging is laborious and provides only temporary benefit.

During the 1890s, an earthworm management method based on a botanical expellant was developed in England¹⁵ and became

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widely used on golf courses.^{16,17} The process involved applying mowrah meal, derived from seeds of the tree *Bassia latifolia* Roxb. after the oil had been pressed out, and liberally watering it, causing earthworms to come to the surface. The worms were raked into piles, shoveled into wheelbarrows and hauled off the site.^{15–18} Other expellant materials, some of which (e.g. mercuric chloride) were highly toxic, were also used on golf courses in the early twentieth century, but proprietary products containing mowrah meal as their active agent were probably the most effective.^{6,16} Mowrah meal contains saponin-glucoside mowrin and other triterpene saponins.^{18,19} Mowrah meal is no longer sold in the United States, nor is it labeled for earthworm management.

Tea seed oil pressed from seeds of the Chinese tea oil plant, *Camellia oleifera* Abel. (Theaceae), is used extensively for cooking in China and elsewhere in eastern Asia, and in the manufacture of soaps, margarine, ointments, hair care products and other items.^{20–22} *Camellia* seeds, fruits and oil are rich in antioxidants²¹ and have long been used in Chinese traditional medicine.²³ Tea seeds, harvested mainly in September and October, traditionally were obtained from wild plants, although cultivation is becoming common (Lee, E., private communication). After the seeds are crushed for oil extraction, the residue is bound together, forming tea seed 'cakes' for easy transportation. Tea seed cake or residue may be ground to a powder or formed into pellets that are used in Asia as an organic molluscicide and, at high rates, to eliminate predatory fish in prawn ponds.^{24,25} Tea seed cake, like mowrah meal, contains triterpene saponins.^{23,26–28} Numerous Chinese websites (e.g. <http://www.camellia-oil.com>) claim it will also eliminate earthworms from golf courses and sports fields. A search of the scientific literature, however, found no references concerning or data supporting tea seed cake for earthworm management.

This study sought to determine if tea seed cake could be used as a botanical pesticide for managing earthworms and reducing casts on turf playing surfaces. Rates of application, the fate of expelled worms and methods for removing them, the effects on other pests and beneficial arthropods and the duration of casting suppression during autumn and spring casting periods were investigated, and the active fraction was identified. Results indicate that tea seed cake is well suited for selective management of earthworm casts on golf courses and sports fields. It could also be useful for reducing bird-attracting earthworm populations at airports.

2 MATERIALS AND METHODS

2.1 General methods

Most field trials were conducted on a 29 × 29 m stand of 'Pennncross' creeping bentgrass *Agrostis stolonifera* L. and annual bluegrass *Poa annua* L. (species distribution, by visual estimation, 75 and 25% respectively) established in 1978 and managed as a push-up golf putting green at the University of Kentucky Turfgrass Research Facility, Spindletop Research Farm, Lexington. The soil is a Maury silt loam (fine, mixed, mesic typic Paleudalf) with pH 6.3. The soil type is homogeneous and original, and no sand topdressing had been applied. The turf was mowed 5 times per week at 4.0 mm height of cut and irrigated from a permanent sprinkler system to prevent visible stress. Nitrogen fertilizer (urea: 46–0–0) was applied in September, October and November at 0.48 kg actual N 100 m⁻² for a total of 1.45 kg N 100 m⁻² per growing season. Fungicides were applied curatively for control of fungal diseases, and dithiopyr was applied as crabgrass control at 0.07 kg AI ha⁻¹. The site had a history of high numbers of earthworm casts, and

there was active casting in the weeks before and during the trials. Different portions of the green were used for each trial.

Tea seed cake pellets (3.2 mm diameter, 5–8 mm long) formulated from fresh *C. oleifera* seed meal that had been harvested and processed as described earlier, without additives, were obtained from Eric Lee (Department of Biology, The Chinese University of Hong Kong) through International Turfgrass Services, Newnan, GA. Powdered tea seed meal was also tested in autumn 2007, but, because the powder was awkward to apply, only the tea seed pellet (TSP) formulation was used in subsequent trials. Analysis by University of Kentucky Division of Regulatory Services, Feed and Fertilizer Laboratory indicated that the TSPs contained 1.2% total nitrogen, 1.3% soluble potash, 0.6% available phosphate and smaller percentages of calcium (0.19), magnesium (0.16), iron (0.11) and trace metals.

2.2 Earthworm expulsion by tea seed cake, the fate of worms and casting reduction after worm removal

A preliminary trial conducted in autumn 2007 evaluated the feasibility of using tea seed cake for earthworm management. Two formulations, powdered tea seed cake and TSPs, were each applied to small (1 × 1 m plots) at three rates, 28, 56 and 112 g m⁻², on 4 October. At that time there was only enough material to treat one replicate with powder and two with TSPs. The formulations were broadcast by gloved hand, evenly covering each plot in two directions, and activated with 1.25 cm of irrigation to the whole site. Worms began surfacing within a few minutes; all those expelled within 30 min were collected into separate cups for each plot, counted and weighed.

Additional trials were initiated in April 2008 to evaluate TSPs for earthworm expulsion, the fate of worms that surfaced and the efficacy in reducing casts. Plot size was 1.5 × 1.5 m, with 0.5 m untreated buffer zones between all plots. The site was hand swept with corn husk brooms immediately before treatment to break up and remove already present casts. Treatments were low, medium or high rates of TSPs (33, 66 and 132 g plot⁻¹ respectively, corresponding to 1.46, 2.93 or 5.86 kg 100 m⁻²), plus untreated controls, arranged in a randomized complete block (RCB) with five replications. Treatments were applied on 9 April; weather conditions were cloudy and 13 °C, and there had been no rainfall or irrigation for the previous 72 h. Immediately after the TSPs were broadcast, a 1 m² area was delineated in the center of each plot by running a string grid from the perimeter. The turf was then irrigated (1.25 cm) and the plots were patrolled by a team of ten persons who stepped only in border areas. All earthworms surfacing within the 1 m² inner portion of each plot within 1 h were hand removed and processed as described below.

The viability of the expelled earthworms was assessed in two ways. First, their ability to burrow down into untreated turf was determined by immediately transferring 20 individuals from each treated plot to one of 20 PVC enclosures (39 cm in diameter by 10.2 cm in height) that had been arranged on an untreated section of the same putting green in the same RCB layout. The worms were evenly placed on the untreated turf, and dead or moribund ones that failed to burrow down within 2 h were counted. Additional samples of 20 surfaced worms per plot were transferred to 947 mL polypropylene containers containing 100 g moistened Magic worm bedding with 10 g Magic worm food (Magic Products, Amherst Junction, WI) and held in darkness at 24 °C for 1 week. Surviving earthworms then were examined, counted and weighed. Very few worms surfaced from the untreated plots, so samples were dug with a spade from an untreated area at the edge of the

same green and used as controls for the enclosure and container assays. Finally, all additional worms that surfaced were collected into separate containers for each plot, counted and collectively weighed.

Another trial conducted on 24 April compared the propensity of expelled earthworms to burrow back down into treated versus untreated turf, an important question if worms are not physically removed from treated areas, or alternatively, are collected and transplanted to golf roughs or other non-treated sites. Five pairs of plots were marked, and one randomly selected plot of each pair was treated with the medium rate of TSPs as described earlier. PVC enclosures, as above, were placed on the treated and non-treated turf. Irrigation was applied, and worms that surfaced within and near enclosures on the treated plots were temporarily removed. Ten representative individuals then were placed on the turf surface within each enclosure and observed. The numbers that remained on the surface, or that had partially or totally burrowed down, were recorded after 1 h.

Analysis of variance (ANOVA) for an RCB was used to test for treatment effects on each variable in the above trials, followed by Dunnett's test ($\alpha = 0.05$) for comparing treatment means with the control, and polynomial contrasts to test for linear or quadratic effects of rate. Counts were log transformed as required to meet the assumptions of normality and homogeneity of variances. Data are reported as original means \pm standard error (SE). Statistical analyses were done with Statistix 8.²⁹

Two independent observers counted the fresh casts on the inner 1 m² of each plot in the main trial at 1, 8, 15 and 35 days after treatment (DAT). Counts were always taken 1–2 days after the green was mowed, to allow at least one night for casts to accumulate. The two observers' counts were averaged per plot, log transformed and examined by ANOVA for repeated measures, followed by ANOVA for main effects and polynomial contrasts for treatment trends on each evaluation date.

2.3 Casting reduction by tea seed cake without worm removal

A trial initiated on 19 April 2008 tested if TSPs would be effective in reducing casts if the expelled earthworms were not immediately removed. Ten 1.5 \times 1.5 m plots, separated by 0.5 m buffers, were marked on a previously unused portion of the push-up green, placing them where similar numbers of abundant casts were observed. All casts were removed with sweep brooms before treatment. Weather conditions were similar and methodology was the same as described in Section 2.2, except that earthworms that surfaced were left on the plots. The numbers of fresh casts on the inner 1 m² portion of each plot were counted by two independent observers at 2 and 9 DAT. The plots were not mowed for 24 h before counting. The two observers' counts were averaged to provide a single value per plot, and then analyzed by ANOVA for repeated measures, followed by two-sample *t*-tests within sample dates.

Another trial tested TSP effectiveness for casting reduction in autumn 2008. Six pairs of 1.5 \times 1.5 m plots were marked on previously unused portions of the push-up green and blocked by pretreatment cast counts. One plot of each pair was treated with 66 g of TSPs per plot, followed by about 1.25 cm of irrigation, on 7 October. Expelled earthworms were left on the plots. Old casts were not removed because they had been compacted by mowing and irrigation. The numbers of fresh casts on the inner 1 m² portion of each plot were counted by two independent observers at 2, 9,

16, 23 and 30 DAT, averaged per plot, and analyzed as above. The plots were not mowed for 24 h before each count.

2.4 Removal of expelled earthworms by mowing or sweeping

Two standard green-keeping practices, mowing and rotary sweeping, were evaluated for removing TSP-expelled earthworms from putting green surfaces. Six pairs of replicated 2 \times 2 m plots were treated with the medium rate of TSPs on 16 April in late afternoon, followed by 1 cm of irrigation. Conditions at the time of treatment were sunny and about 20 °C. Expelled worms were left on the plots, and the following morning there were numerous dead or moribund earthworms on the turf surface. The locations of 15 earthworms on each plot were then marked by dabbing a small spot of orange turf marking paint on the grass beside the cadaver. The worms themselves were not disturbed, except for one plot where only three worms had surfaced, so 12 additional earthworms from other treated plots were placed on that plot.

One plot of each pair was then mowed once with a Toro Greensmaster 3150 riding mower (Toro Company, Bloomington, MN) with nine-bladed cutting units set at 4 mm height of cut, and the number of marked worms removed from each plot was recorded. The second set of plots was swept with an Agri-Fab rotary broom sweeper (model 45-0320-062; Agri-Fab, Sullivan, IL) pulled by a Gator utility vehicle (John Deere, Lathrop, CA). The brushes were adjusted to contact the surface and had an effective sweeping width of 1.1 m. The numbers of marked earthworms remaining were recorded after the first pass and again after a second pass from the opposite direction. The percentage of earthworms removed was compared between the two methods by paired *t*-tests.

2.5 Potential impact on pest insects and non-target soil arthropods

Given the anecdotal reports that tea seed cake has insecticidal activity, the authors sought to determine if applying TSPs for managing earthworms and casts might also control two common turfgrass pests: foliage-feeding black cutworms, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), and root-feeding white grubs (Coleoptera: Scarabaeidae). The effects on soil microarthropod populations were also evaluated.

Six pairs of polyvinyl chloride (PVC) cylinder enclosures (39 cm diameter, 10.2 cm height, 0.12 m² enclosed area) were driven about 7 cm deep into a stand of 'Penncross' creeping bentgrass turf adjacent to the main study site. The turf was managed similarly to golf course fairways, i.e. fertilized with 146 kg N ha⁻¹ year⁻¹ from urea, irrigated from a permanent sprinkler system to prevent drought stress and mowed at 16 mm 3 times per week. Twenty-five third-instar *A. ipsilon* shipped from a commercial insectary were added to each enclosure in the morning on 13 May, and those few larvae that had not burrowed into the turf within 5 min were replaced. The cutworms were allowed to establish for 5 h, then TSPs were applied to one ring of each pair at the aforementioned high rate (131.5 g 1.5 m⁻²). Control enclosures were not treated. Irrigation (1.25 cm) was then applied to the whole site, and pieces of mesh wire hardware cloth were secured over the enclosures to prevent bird predation. Survival was assessed after 72 h by sampling each enclosure by soap drench [10 mL Joy Ultra dishwashing detergent (Proctor and Gamble, Cincinnati, OH) per 7.6 L water], which causes healthy cutworms to surface.

Impact on white grubs was assessed by driving ten pairs of 25 cm diameter, 20 cm high beveled PVC rings 17 cm deep into a

stand of 'Midnight' Kentucky bluegrass, *Poa pratensis* L., near the other study sites. Third-instar masked chafers (*Cyclocephala* spp.) were dug from untreated roughs of a local golf course; 14 grubs were placed in each enclosure on 2 May. The grubs were allowed 48 h to establish before one enclosure of each pair was treated with the high rate of TSPs, followed by irrigation as described above. The turf and soil within each enclosure was broken apart and examined on 13 May, and remaining grubs were counted. Viability was assessed by placing grubs on the surface of moistened peat moss and recording the percentage able to burrow beneath the surface, the normal response, within 10 min.

Impact of TSPs on soil arthropods in the creeping bentgrass push-up green was assessed by pulling two cores (5.08 cm diameter, 5.08 cm deep) of grass and soil from each plot in the first April 2008 trial (Section 2.2). The samples were taken 4 weeks after application, cut into eight smaller pieces, to reduce the chances of microarthropods being trapped in the drying soil, and extracted for 72 h in Tullgren funnels (Burkhardt Agronomic Instruments, Uxbridge, Middlesex, UK) equipped with 25 W bulbs. Samples were stored in 75% ethanol until sorted. Predominant taxa, mainly oribatid and mesostigmatid mites and Collembola, were counted with a binocular microscope and compared among treatments as described for the earthworm counts.

TSP impact on soil microarthropods in fairway-cut creeping bentgrass was assessed in the same stand as that used for the black cutworm trial. Paired PVC enclosures (39 cm diameter) were set on the turf, and the high rate of pellets was applied within one enclosure of each pair on 10 May, followed by 1.25 cm of irrigation to the whole site. Two cores of grass and soil were pulled from each plot after 72 h and extracted in Tullgren funnels as described above. Paired *t*-tests ($P < 0.05$) were used to compare means for treated and control plots in this assay and others described in this section.

2.6 Determination of earthworm-active components in TSPs

Bioassay-guided extraction and fractionation were done to identify the active vermicide components in the TSPs. The bioassays used freshly collected earthworms (*Aporectodea* sp.) dug at the main study and placed on moist filter paper in 90 mm diameter petri dishes, with 3–5 worms per plate and 4–6 replicates per treatment. Each trial included a positive control (a suspension of 187 mg TSPs ground with a mortar and pestle and dissolved in 32 mL water), which corresponded to the medium rate (65.8 g TSPs 2.24 m^{-2}) used in field trials, and a water control. Extracts and fractions were diluted in distilled water and applied via pipette directly to the earthworms, which were observed for immediate response (e.g. agitation, curling) and prodded to evaluate mortality or morbidity after 1 and 2 h.

For the bioassay-guided fractionation, crushed TSPs (500 g) were extracted twice with 1.5 L methanol by stirring the suspension for 2 h. The extracts were filtered through Whatman No. 1 filter paper, and the solvent was evaporated to yield 53 g of extract which was screened, along with the residual powder after extraction, for activity. Further, the methanol extract was partitioned between water and butanol (1 : 1), using 30 mL butanol 2.5 g^{-1} . The aqueous layer was lyophilized, and the butanol soluble fraction was evaporated, resuspended in distilled water and assayed. The active butanol-soluble fraction of the methanol extract was run on a gravity column to purify the compound, with activity confirmed by bioassay. This active fraction was subjected to silica gel column chromatography by means of a Biotage flash column chromatography system using 50% ethyl acetate in

hexane, 100% ethyl acetate and 20% methanol in ethyl acetate, with 0.5% (v/v) water. Fractions of 18 mL were collected, and similar fractions according to the TLC profile were combined to afford six fractions. Each fraction was tested in the bioassay to identify the active fraction. Two fractions were found to have the highest activity. These two fractions were further purified by silica gel column chromatography to afford an off-white powder (112 mg). ^1H and ^{13}C NMR spectra of the active samples were obtained on 400 and 600 MHz instruments.

Saponin concentration in the TSPs was estimated from its hemolytic activity^{30,31} using commercially available *Gypsophila* sp. (soapwort) saponins (Sigma #1252; Sigma-Aldrich, St Louis, MO) as a standard. For standard curves, four solutions (0.2, 0.33, 0.5 and 1 mg mL^{-1} saponins in 0.9 M sodium chloride) were prepared; then aliquots (0, 5, 10, 25, 50, 75 or 100 μL) of each solution were added to separate wells of 96-well, V-bottom, uncoated titration plates (Costar, Cambridge, MA). Corresponding amounts of 0.9 M sodium chloride buffer were added to bring the volume to 100 μL in each well. TSPs were defatted with hexane and extracted twice with ethanol, with the solvent evaporated under nitrogen, and the precipitate was resuspended in 0.9 M sodium chloride. Ten serial dilutions of the TSP extract (0–67% in 0.9 M sodium chloride) were added to well plates. Fresh rabbit blood (100 μL) was added to each well; plates with TSP extract or saponin standard were read after 4 h by determining the threshold required to induce hemolysis. The saponin content of three samples of TSPs was estimated by comparing hemolysis thresholds of TSP extract with the standards.

3 RESULTS

3.1 Earthworm expulsion by tea seed cake, the fate of worms and casting reduction after worm removal

Both the powdered tea seed cake and the TSPs expelled a large number of earthworms in the October 2007 preliminary trial on the push-up green. Worms began surfacing from treated plots a few minutes after irrigation. Totals of 93, 63 and 61 worms m^{-2} were expelled from the non-replicated plots treated with the low, medium and high rates of powder. For the two replicates treated with TSPs, mean \pm SE numbers of worms expelled were 59 ± 13 , 55 ± 9 and $56 \pm 10 \text{ m}^{-2}$ respectively. Individual earthworms were not identified, but the species complex at this site is dominated by smaller species, mainly *Aporectodea* spp., that make shallow, semi-permanent burrows and deposit casts on the surface.³² Thousands of these earthworms were expelled by the treatments, as opposed to very few night crawlers, *Lumbricus terrestris* L. The powder formulation was dusty, prone to drift and awkward to apply, so only TSPs were used in subsequent trials.

In April 2008, all rates of TSPs followed by irrigation rapidly expelled a large number of earthworms, whereas almost none surfaced in response to irrigation alone (Table 1). Worms expelled by the two highest rates showed impaired ability to burrow down when placed on untreated plots on the same green, and their survival when transferred to clean worm bedding was also compromised (Table 1). Many of the affected earthworms appeared flaccid, with reddish cankers. All of the aforementioned variables showed significant linear and quadratic response to application rate (polynomial contrasts, $P < 0.05$).

All rates of TSPs also greatly reduced the numbers of earthworm casts for at least 35 days when counting was terminated (Fig. 1). Repeated-measures ANOVA indicated significant effects

Table 1. Mean (\pm SE) number per 1 m^2 , total mass and viability of expelled earthworms following the application of TSPs to a push-up creeping bentgrass green, April 2008^a

Rate (g m^{-2})	Worms expelled m^{-2}		Viability in untreated medium	
	Number	Mass (g)	% able to burrow down	% survival after 1 week
0	0(\pm 0)	0(\pm 0)	100(\pm 0)	95(\pm 2)
14.6	172(\pm 31)*	20(\pm 4)*	94(\pm 4)	71(\pm 6)
29.2	216(\pm 48)*	29(\pm 5)*	84(\pm 6)*	44(\pm 10)*
58.4	229(\pm 38)*	32(\pm 8)*	74(\pm 10)*	46(\pm 17)*

^a ANOVA results: $F = 5.0, 10.2, 6.0$ and 6.1 for number, mass, % able to burrow and % survival respectively; $df = 3, 12$; $P < 0.01$ for each variable. Within columns, means marked with an asterisk (*) differ significantly from the corresponding control mean (Dunnett's test, $\alpha = 0.05$).

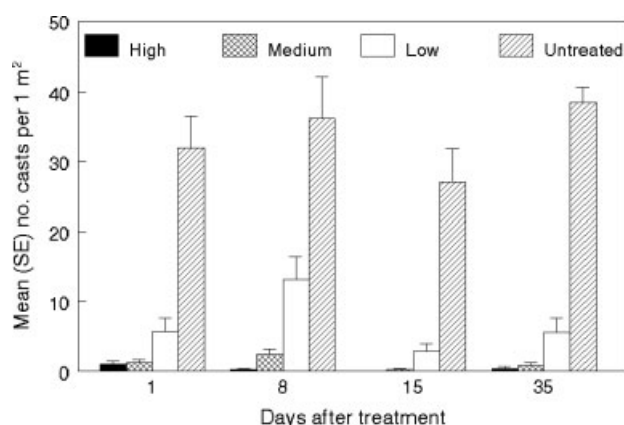


Figure 1. Reduction in earthworm casts following TSP application on 9 April 2008 and physical removal of worms that surfaced. High, medium and low rates of TSPs were 5.86, 2.93 and 1.46 $\text{kg } 100\text{ m}^{-2}$ respectively.

for treatment ($F = 46.6$; $df = 3, 16$; $P < 0.001$), date ($F = 3.72$; $df = 3, 9$; $P < 0.05$) and treatment by date interaction ($F = 2.76$; $df = 9, 46$; $P < 0.05$). Reductions were also significant within each sample date ($F = 39.8, 27.0, 29.2$ and 159 for 1, 8, 15 and 35 DAT respectively; $df = 3, 12$; $P < 0.001$), with linear and quadratic effects for rate (polynomial contrasts; all $P < 0.001$, except for the quadratic trend at day 8, which was significant at $P < 0.05$). Reductions in casts from the low, medium and high rates averaged 89.6, 99.3 and 100% respectively at 15 DAT, and 85.4, 98 and 99% respectively at 35 DAT.

In the second reburial trial, expelled earthworms were more likely to die on the surface if left on treated plots than if transferred to untreated plots on the same green. Mean (\pm SE) numbers (out of 10) remaining wholly exposed after 30 min were 6.2 ± 1.3 versus 1.8 ± 0.4 for expelled worms transferred to treated versus untreated turf respectively ($t = 3.30$; $df = 4.7$; $P = 0.01$; one-tailed, two-sample t -test with correction for unequal variances).²⁶ If expelled worms that had burrowed only part-way down were included, then the numbers that had failed to rebury were 6.8 ± 1.0 versus 3.0 ± 0.5 , respectively, for worms placed on treated or untreated plots ($t = 3.28$; $df = 8$; $P < 0.01$).

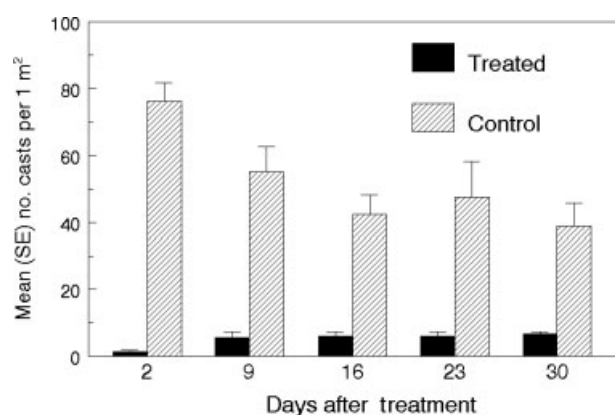


Figure 2. Reduction in earthworm casts following application of TSPs ($2.93\text{ kg } 100\text{ m}^{-2}$) on 7 October 2008, with expelled worms left on the surface.

3.2 Casting reduction by tea seed cake without worm removal

The application of TSPs on 19 April greatly reduced numbers of earthworm casts, even when expelled earthworms were not physically removed. At 2 DAT the numbers of casts on treated plots were reduced by 99% compared with the controls ($1.4 \pm 1.0\text{ m}^{-2}$ versus $120 \pm 24\text{ m}^{-2}$ respectively; $t = -9.93$, $df = 8$, $P < 0.001$; analysis on log-transformed data). At 9 DAT there were still almost no casts on the treated plots compared with the controls ($0.4 \pm 0.2\text{ m}^{-2}$ versus $15.6 \pm 2.7\text{ m}^{-2}$ respectively; $t = -10.9$, $df = 8$, $P < 0.001$). The night preceding 28 April was unseasonably cool (low = 6.7°C , with an average low for the preceding 8 days of 11.7°C), which probably accounts for the relatively low numbers of casts on the control plots at the 9 DAT evaluation. Application in early October was similarly effective, reducing casts by 98.3, 89.7, 85.8, 87.4 and 82.8% after 2, 8, 16, 23 and 30 days respectively (Fig. 2).

3.3 Removal of expelled earthworms by mowing or sweeping

A single pass with the greens mower or sweeper 1 DAT removed about 66 and 40% of the dead and moribund earthworms present on the putting green surface. The numbers of marked worms (out of 15) remaining on the plots after a single pass averaged 5.2 ± 0.7 versus 9.0 ± 1.9 for mowing and sweeping respectively ($t = 2.04$; $df = 9$; $P = 0.07$). Sweeping a second time in a perpendicular direction removed a few additional worms, increasing the cumulative removal from those plots to about 48%, leaving a mean of 7.8 ± 1.8 worms still present. The one replicate for the sweep treatment on which earthworms had been placed to augment their numbers was excluded from the analysis because the sweeper was much more effective in removing those earthworms (13/15 removed by the first pass) than in removing dead earthworms that had not been disturbed. It was observed that secretions from earthworms that died *in situ* caused them to adhere to the grass and be less efficiently dislodged by the sweeper than individuals that had been moved.

3.4 Potential impact on pest insects and non-target soil arthropods

Application of TSPs followed by irrigation failed to reduce the abundance of black cutworms in fairway-height creeping bentgrass or of masked chafer grubs in Kentucky bluegrass turf (Table 2). Larvae sampled from the treated plots were vigorous and

Table 2. Absence of TSP effect on implanted white grubs (*Cylocephala* spp.) and black cutworms (*Agrotis ipsilon*) and natural populations of non-target soil microarthropods in creeping bentgrass turf

	Mean number per sample (\pm SE)		
Taxons sampled	Treated	Control	<i>t</i> (<i>P</i>) ^a
Pest insects^b			
Masked chafer grubs	10.8(\pm 0.7)	12.2(\pm 0.4)	−1.7 (0.06)
Black cutworms	20.5(\pm 0.8)	20.8(\pm 0.9)	−0.3 (0.40)
Soil microarthropods, push-up green^c			
Oribatid mites	119.0(\pm 12.9)	104.6(\pm 18.5)	0.7 (0.52)
Mesostigmatid mites	34.6(\pm 6.1)	25.8(\pm 6.5)	1.1 (0.34)
Collembola	30.2(\pm 2.5)	25.8(\pm 6.0)	0.8 (0.49)
Root aphids	7.6(\pm 2.1)	17.4(\pm 8.1)	−1.4 (0.23)
Diplura	3.0(\pm 1.1)	1.2(\pm 0.5)	1.6 (0.19)
Protura	1.6(\pm 1.0)	0.4(\pm 0.4)	1.2 (0.28)
Midge larvae	1.6(\pm 1.4)	0.2(\pm 0.2)	1.0 (0.38)
Soil microarthropods, fairway-height turf^c			
Oribatid mites	55.0(\pm 11.3)	55.0(\pm 14.0)	0.0 (1.0)
Mesostigmatid mites	69.0(\pm 3.4)	59.5(\pm 5.8)	1.3 (0.25)
Collembola	37.0(\pm 13.5)	38.3(\pm 6.1)	−0.1 (0.93)
Root aphids	3.3(\pm 2.6)	1.6(\pm 1.3)	1.8 (0.13)
Midge larvae	3.5(\pm 0.5)	2.5(\pm 1.0)	0.7 (0.50)

^a Paired *t*-tests, treated versus control; df = 5, 4 and 5 for pest insects and soil microarthropods in push-up green or fairway turf respectively.

^b For masked chafers, 14 grubs were established in each plot, treated and sampled 9 DAT. Black cutworm plots were infested with 25 third instars, treated and sampled after 72 h. Data analyzed by two-sample *t*-tests.

^c Counts based on Tullgren funnel extractions of two 5.08 cm diameter, 5.08 cm deep cores of soil and thatch. Putting green and fairway-height plots sampled 4 weeks or 72 h after treatment respectively.

appeared normal. Similarly, TSP application to creeping bentgrass maintained as a push-up green, or at fairway height, did not affect the abundance of any of the major taxa of soil microarthropods in either setting (Table 2).

3.5 Determination of earthworm-active components in TSPs

The methanol extract of TSPs caused immediate irritation (e.g. rapid crawling, curling with a whipping motion, copious mucus production) and 100% morbidity or mortality of topically treated earthworms within 2 h. The same effects occurred with the TSP standard. The residual powder after methanol extraction was not active. Subsequent bioassays showed high activity in the butanol-soluble fraction of the methanol extract, with topically treated earthworms showing immediate irritation and mucus production, followed by cuticle lesions, apparent cell lysis, leakage of blood and 100% mortality within 2–3 h. The NMR spectra of this active fraction were typical for saponins. Tea saponins contain highly oxygenated aglycones. Owing to extensive overlap in the NMR resonances for oxygenated carbons (in the ¹³C NMR) and the proton regions (¹H NMR) in the NMR spectra, unambiguous identification of aglycones was not possible. The authors could locate at least three sugar units, evidenced by signals responsible for anomeric carbons in the ¹³C NMR. Based on hemolytic activity, the crude TSP material contained an estimated 12% saponins.

4 DISCUSSION

This study indicates that tea seed cake, the saponin-rich residue of *C. oleifera* seeds after the oil is extracted, is an effective botanical vermicide that could be used selectively to manage earthworms and their casts on golf courses and sports fields. The rate of TSPs (2.93 kg 100 m^{−2}) that provided >98% reduction in casts by 2–3 DAT, and 83–98% reduction after 4–5 weeks, is 60% lower than the rate at which mowrah meal was used in the past.^{6,16} Also, the relatively light amount of irrigation (\leq 1.5 cm) needed to activate TSPs would lessen risks of run-off from treated sites compared with the profuse watering that was needed with mowrah meal.^{16,17} No phytotoxicity or turf discoloration was apparent at the rates of TSPs that were applied. TSPs were readily available as a byproduct of *Camellia* oil manufacture and should be relatively inexpensive to market and use. TSPs could also be used to sample earthworms in ecological studies, which in the past have typically involved drenching with dilute formalin,³³ a known carcinogen, and more recently with powdered mustard seed.⁸

Several forms of saponins, which contribute to the bitterness of tea, have been isolated from *Camellia* seeds, leaves and roots.²³ The present estimate of 12% saponins in the TSPs used in this study is similar to reports of 13.5% saponins in *C. oleifera* fruit hulls²² and >10% in the seeds,³⁴ but higher than reported concentrations in Thai and Japanese tea seeds (5.2 and 7.2% respectively).²⁴ No previously published studies concerning the toxicology of tea seed cake or *Camellia* saponins to earthworms were found, but saponins isolated from stems of a cactus, *Opuntia vulgaris* Mill., exhibited dose-dependent activity, including paralysis and death, on the Indian earthworm *Pheretima posthuma* Vaill.³⁵ Although the tea plant also produces polyphenols and alkaloids,³⁶ bioassay-guided fractionation indicates that the vermicial activity of TSPs results from a mix of closely related saponins. The authors are carrying out structure elucidation work to identify those specific saponins.

In China, *Camellia* seed residue is reputed to protect crops from certain aphids, scales, leafhopper, beetles and caterpillars,²² but a literature search found no published efficacy data for such usage. In laboratory tests, saponins refined from tea seed suppressed population growth of two spider mite (Tetranychidae) species, and concentrated sprays also reduced oviposition and killed larvae and nymphs, but not adult mites.³⁷ Ethanolic extract of tea seed cake also had contact activity against Pharaoh ant, *Monomorium pharaonis* L., workers on filter paper.³⁸

It was found, however, that a rate of TSPs that expelled and killed earthworms did not measurably impact on black cutworms, masked chafer grubs or soil microarthropods in creeping bentgrass turf. This indicates that TSP components either lack contact activity against those arthropods or else did not reach the thatch and soil at high enough concentration for activity to be expressed. Saponins deter feeding by various non-adapted arthropods,^{30,39} but effects of TSPs on feeding by cutworms or grubs were not evaluated in the present study. Samples suggest that the use of TSPs on greens or sports fields would not disrupt beneficial soil invertebrates other than earthworms.

Plant-derived toxicants, including tea seed saponins, are used in China, Taiwan, Thailand and other East-Asian countries to eradicate undesirable fish in shrimp farming ponds.^{24,25} Saponins act on the respiratory epithelium of fish gills, causing oxygen deprivation.^{40,41} Tea seed saponins degrade naturally in the environment in 2–10 days, and at low concentrations are less toxic to shrimps, crabs and other food organisms in the ponds.^{24,42} Sublethal exposure can, however, have chronic effects on decapod

crustaceans, e.g. impaired feeding and growth, shell softening and increased susceptibility to pathogens.^{43,44}

Toxicity of tea seed saponins to fish could be an issue in its registration for use in managing earthworm casting on golf courses. Typical rates for tea seed saponins or cake used in eastern Asia for eradicating undesirable fish in shrimp ponds are 1.1 and 15 mg L⁻¹ respectively.^{24,42} The 24 h LC₅₀ for tea seed cake to Nile tilapia (*Oreochromis niloticus* L.) is about 6 mg L⁻¹.²⁵ By comparison, lambda-cyhalothrin or bifenthrin, the active ingredients in pyrethroid insecticides labeled for use on putting greens, have 96 h LC₅₀ values to bluegill sunfish of 0.00035 and 0.00021 mg L⁻¹ respectively,⁴⁵ corresponding to about 1.1 and 0.85 mL L⁻¹ of finished spray at a medium labeled tank mix rate (11.8 mL product 3.785 L⁻¹). A typical 3 m deep, 0.3 ha (9000 m³) golf course pond would need to be contaminated by 54 kg of tea seed cake in order to reach 6 mg L⁻¹. With label restrictions specifying buffer zones around ponds or streams, it should be possible to use TSPs with little or no hazard to aquatic organisms.

A disadvantage of earthworm expellants such as mowrah meal used in the past was that collection of expelled earthworms was unpleasant and labor intensive, and disposing of them could be problematic.⁶ Most of the earthworms expelled by TSPs, however, die on the surface, where they desiccate relatively quickly and can be removed by mowing or sweeping. Dried worm cadavers remaining on plots were hardly noticeable by the second day. The ideal remedy would provide economical, long-lasting suppression of casting without killing the earthworms, but such a remedy has not yet been found.

Earthworms in grass aprons of runways and taxiways or moving onto pavement after rains can become an aviation hazard by attracting birds, increasing the risk of bird strikes during take-off and landings, and by creating slippery conditions for aircraft rolling over them.^{10–12} The fungicides benomyl and thiram, which were formerly used to control earthworms on such sites, are no longer available. Earthworm control is one of the habitat modification practices recommended by the USA Federal Aviation Administration's guidelines for bird strike mitigation.⁴⁶ TSPs could be useful for that purpose.

Before 1978, widespread usage of organochlorine biocides (e.g. chlordane) against turf insect pests typically suppressed earthworms and casting as a side effect. A number of pesticides used on turf in the 1980s and 1990s were also toxic to earthworms^{33,47} and sometimes used off-label to suppress casting, but registrations of most of those compounds have been cancelled. No pesticides are labeled for earthworm control in the United States. This study indicates that TSPs are far more effective than any other method (e.g. abrasive topdressings, acidifying fertilizers, removal of clippings) being used to manage casts on golf courses or sports fields. With appropriate label restrictions specifying buffer zones around water, TSPs or other saponin-rich byproducts of tea oil manufacture could be useful for selective management of excessive earthworm casts on closely mowed playing surfaces.

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